Supplemental Table 1. Skeletal analysis of AER-FGF mutants

	Genotype	n (limbs)	Zeugopod No. of Elements		Autopod Digit Number			
			2	1	5	4	3	2
FL	Control	28	28/28 (100%)		28/28 (100%)			
	F8-KO	20	20/20 (100%)		1/20 (5%)	19/20 (95%)		
	F8-KO; $F9^{-/+}$	12	12/12 (100%)		2/12 (17%)	8/12 (67%)	2/12 (17%)	
	F8;9-DKO	32	7/32 (22%)	25/32 (78%)		22/32 (69%)	9/32 (28%)	1/32 (3%)
HL	Control	28	28/28 (100%)		28/28 (100%)			
	F8-KO	20	17/20 (85%)	3/20 (15%)		20/20 (100%)		
	F8-KO; $F9^{-/+}$	12	10/12* (83%)	2/12 (17%)		12/12 (100%)		
	F8;9-DKO	32	1/32 (3%)	31/32 (97%)		32/32 (100%)		
			Stylopod – Autopod (no zeugopod)			Stylopod only		No limb
FL	F8;4-DKO; $F9^{-/+}$	28	22/28 (79%) (large gaps: 7/22 [27%])			6/28 (21%)		
HL	F8;4-DKO; $F9^{-/+}$	28						28/28 (100%)
FL	F8;4;9-TKO	18						18/18 (100%)
HL	F8;4;9-TKO	18						17/18^ (94%)

*very short

^1/18 (6%) with femur, tibia and digit elements

Figure S1. Analysis of *Hoxa11* expression in AER-FGF compound mutant limb buds.

At E12.5, assays for *Sox9* expression demonstrated that zeugopod elements were missing in F8;F9-DKO and F8;F4 DKO;F9$^{-/+}$ forelimbs (see Fig. 2j,k). *Hoxa11* is thought to mark zeugopod elements at late stages[1,2], so we sought to determine if zeugopod progenitors were present earlier in mutant limb buds of the genotypes indicated by whole mount RNA in situ hybridization with a probe for *Hoxa11*. In F8;F9-DKO forelimb buds at E11.5, we detected *Hoxa11*-expressing cells in an A-P stripe. This was unexpected because a day later the condensation that develops into the radius (anterior zeugopod) is absent (Fig. 2j). *Hoxa11*-expressing cells were likewise detected at E11.5 in an A-P stripe in F8;F4 DKO;F9$^{-/+}$ forelimb buds, which at E12.5 completely lack zeugopod elements (Fig. 2k). However, since we also found that most of the *Hoxa11*-positive cells in control limb buds at E11.5 appeared to reside in the proximal autopod (arrowheads), it seems that *Hoxa11* is not useful as a marker for zeugopod elements at this stage, and the identity of the *Hoxa11*-positive cells in the mutant limb buds is uncertain.

Figure S2. Model explaining how AER-FGF mutant phenotypes are generated.

Diagrams depict dorsal views of mouse limb buds and illustrate how the dual function of AER-FGFs (blue) as survival factors and distalizing signals influences skeletal development. Limb bud development from the early bud through the condensation stages, which occur in proximal to distal sequence, is shown from left to right, with the skeletal phenotype on the far right. (a) In wild-type limb buds, the two-signal dynamic specification model proposes that specification of the P and D domains that will develop into stylopod (S, tan) and autopod (A, purple) occurs at an early stage, and that specification of the middle domain (Z, gold) occurs slightly later. The period of specification is followed by a period of expansion of the specified cells, during which S, Z, and A progenitors condense sequentially (indicated by stippling). (b) Initially, the F8-KO;F9$^{-/+}$ mutant forelimb bud is of normal size, because *Fgf8* is transiently expressed before it is inactivated[3,4] (dark blue with light blue cross-hatching). However, once *Fgf8* expression is extinguished in the absence of one copy of *Fgf9* (light blue), there is abnormal cell death (represented by red dots). Consequently, the mutant limb bud is smaller than normal and subsequently does not reach normal size. According to the two-signal dynamic specification model, the decrease in distalizing signal (i.e. AER-FGF signaling) results in a larger than normal

S domain, whereas A and Z domains are smaller than normal. Some of the S progenitors are presumably removed before condensation by the abnormal proximal cell death that results from reduced AER-FGF signaling, resulting in an S (humerus) that is slightly smaller than normal, and a Z and A that are slightly reduced. (c) In contrast, initially the F8-KO;F9$^{-/+}$ mutant hindlimb bud is smaller than normal because *Fgf8* is never expressed[3,4], and remains so throughout limb development. The model proposes that during P-D specification, as in the forelimb, the Z and A domains are reduced. However, the S domain is also smaller than normal because of the decrease in limb bud size, and is reduced even further by the abnormal proximal cell death due to decreased AER-FGF signaling. Ultimately, since S progenitors have less time than Z and A progenitors to expand before condensation occurs, the S (femur) is more severely compromised than Z and A elements.

Similar logic can be used to explain the phenotype observed when exposure to X-irradiation is used to kill cells throughout the chicken limb bud[5]. In such cases, even though P- and D- signals are normal, the extent of the domains these opposing signals specify are necessarily smaller than normal because of the reduction in limb bud size. Thus, all skeletal elements should be reduced. However, because irreversible determination occurs in a proximal to distal sequence, stylopod progenitors have less time to expand before they begin differentiation than zeugopod/autopod progenitors. Therefore, the stylopod is more severely compromised than zeugopod/autopod.

References cited

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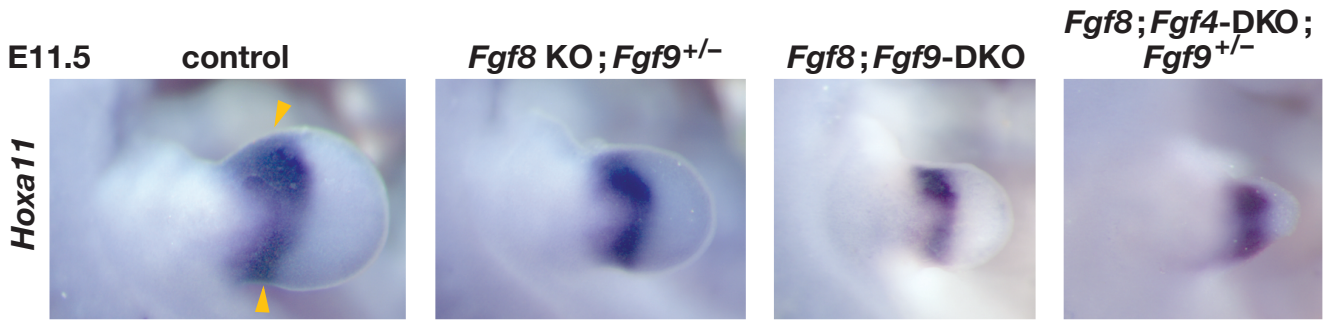
Supplemental Figure 1



Supplemental Figure 2.